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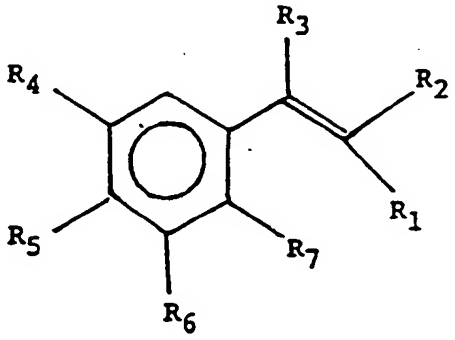
(54) **Benzylidene- and cinnamylidine-malononitrile derivatives for the inhibition of proliferative processes in mammalian cells.**

(57) There are provided pharmaceutical compositions containing as an active ingredient a compound of the general formula (I):



(I)

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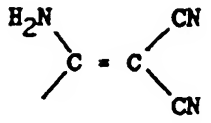
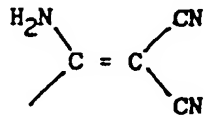
wherein R_1 and R_2 are each independently CN, $CONH_2$ or COOH or one of R_1 ad R_2 may be $-CSNH_2$ or, when R_1 is CN, R_2 can also be the group



R_3 is H, CH_3 or OH,

R_4, R_5, R_6, R_7 are each independently H, OH, C_{1-5} alkyl, C_{1-5} alkoxy, NH_2, CHO, halogen, NO_2 or COOH, or R_4 and R_5 together may represent a group $-O-CH_2-O-$;

provided that: (a) when R_4 ad R_7 are each OH, R_3, R_5 and R_6 are each H and one of R_1 and R_2 is CN, then the other of R_1 and R_2 cannot be $CONH_2$; and (b) when R_3 and R_7 are each H, R_5 is OH and R_4 and R_6 are both H or both C_{1-5} alkyl, then R_1 is CN and R_2 is CN or the group



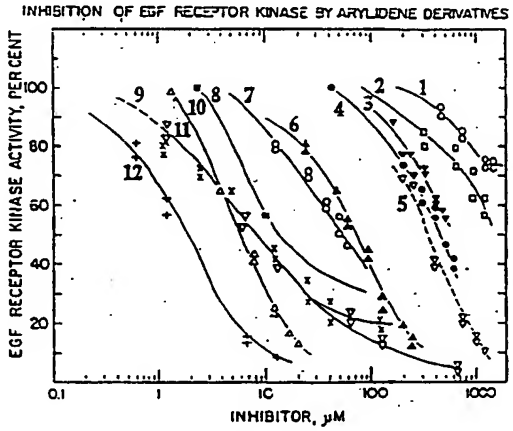
;

or a pharmaceutically acceptable salt thereof.

There are also provided some novel compounds of formula (I) above.

The compositions and compounds according to the invention are efficient protein-tyrosine kinase inhibitors and are suitable for the inhibition of proliferative processes in mammalian cells.

FIGURE 1



COMPOUND	SUBSTITUENTS							Kinh μM
	R_3	R_4	R_5	R_6	R_7	R_2	R_1	
1	H	H	OH	H	H	CO_2H	H	1000
2	H	H	OH	H	H	CO_2H	CO_2H	500
3	H	H	OH	H	H	CN	CN	166
4	H	OH	OH	H	H	CO_2H	H	150
5	H	H	H	OH	H	CN	CN	123
6	H	OH	H	H	OH	CN	CO_2H	24
7	H	H	OH	OH	H	CO_2H	CN	18
8	H	H	OH	OH	H	CN	CN	11
9	H	OCH_3	OH	OH	H	CN	CN	2
10	H	OH	OH	OH	H	CN	CN	1
11	H	H	OH	OH	H	$CONH_2$	CN	2.3
12	H	H	OH	OH	H	$CSNH_2$	CN	0.85

FIELD OF INVENTION

The present invention concerns novel pharmaceutical compositions containing substituted benzylidene- and cinnamylidene-malononic acid derivatives for specifically inhibiting cell proliferation processes in mammals. The invention further provides certain novel compounds of the aforesaid type.

BACKGROUND OF THE INVENTION

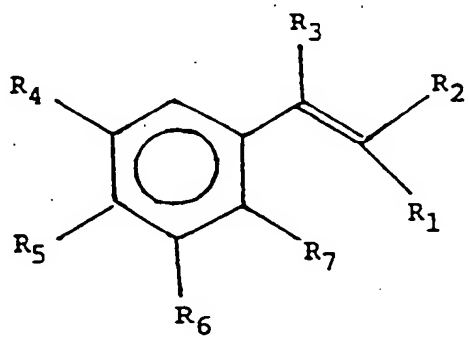
Currently the chemotherapy of cancer makes use of inhibitors of DNA synthesis (examples: adriamycin, fluorouracil) and compounds which disrupt the cytoskeleton (vinblastine). These compounds are highly toxic since their inhibitory activity is not limited to cancer cells, with the distinction, however, that tumor cells are more readily attacked by the aforesaid inhibitors because these cells divide more rapidly and their DNA metabolism is consequently more active. A few types of cancers are nowadays treated with specific pharmaceutical agents. These include, for example, certain breast cancers which are hormone dependent and can therefore be treated with specific hormone derivatives. These cases, however, are the exception and the chemotherapeutic treatment for the majority of the various types of cancer is non-specific.

In the early 1980's it became apparent that 20 to 30 percent of cancers express characteristic oncogenic products which are growth factor receptors or their mutated homologs, which exhibit protein tyrosine kinase (PTK) activity. The PTK activity is intrinsic to the receptor or its oncogene homolog and, which influences the cell proliferation via its PTK domain. Furthermore, each of these receptors (normal or mutated), exhibits a characteristic PTK activity with a distinct substrate specificity. One of these receptors is the epidermal growth factor (EGF) receptor and its oncogenic homolog v-Erb-b. Pursuant to that discovery it has already been proposed to treat cancer by means of various chemical substances capable of inhibiting the PTK activity of EGF - see, for example, in Japanese patents Nos. 6239523, 6242923 and 6242925.

It is the object of the present invention to provide readily accessible compounds of relatively simple structure that are active as specific EGFR-tyrosine kinase inhibitors and can thus serve as specific anti-cancer agents.

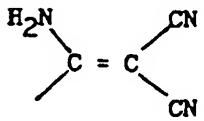
GENERAL DESCRIPTION OF THE INVENTION

The above object is achieved by the present invention which provides pharmaceutical compositions containing as an active ingredient a compound of the general formula (I):



(I)

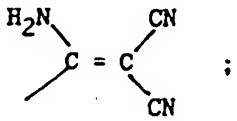
wherein R_1 and R_2 are each independently CN, $CONH_2$ or COOH or one of R_1 and R_2 may be $-CSNH_2$ or, when R_1 is CN, R_2 can also be the group



R_3 is H, CH_3 or OH,

R_4, R_5, R_6, R_7 are each independently H, OH, C_{1-5} alkyl, C_{1-5} alkoxy, NH_2, CHO, halogen, NO_2 or COOH,

or R_4 and R_5 together may represent a group -O-CH_2-O-;
provided that: (a) when R_4 and R_7 are each OH, R_3, R_5 and R_6 are each H and one of R_1 and R_2 is CN, then the other of R_1 and R_2 cannot be $CONH_2$; and (b) when R_3 and R_7 are each H, R_5 is OH ad R_4 and R_6 are both H or both C_{1-5} alkyl, then R_1 is CN and R_2 is CN or the group



or a pharmaceutically acceptable salt thereof.

Preferred pharmaceutical compositions are those comprising an active ingredient of formula I in which at least one of R_1 and R_2 is CN cis to the phenyl moiety of said formula.

Amongst the preferred compositions, more preferred are those comprising an active ingredient in which R_4 and R_5 are hydroxy groups, R_6 is hydrogen or hydroxy and R_3 and R_7 are hydrogens.

Especially preferred pharmaceutical compositions are those containing as an active ingredient a compound selected from:
3,5-dihydroxybenzylidene-malononitrile,
α-hydroxy-3,4,5-trihydroxybenzylidene-malononitrile,
3-methoxy-4,5-dihydroxybenzylidene-malononitrile,
α-cyano-3,4-dihydroxycinnamthioamide,
α-cyano-3,4-dihydroxy-cinnamamide,
3,5-di-t-butyl-4-hydroxybenzylidene-malononitrile,
4-formylbenzylidene-malononitrile,
4-hydroxybenzylidene-malononitrile,
3,4-methylenedioxy-6-nitrobenzylidene-malononitrile,
3,4-dihydroxybenzylidene-malononitrile,
3,4,5-trihydroxybenzylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malononitrile, and
γ-cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile; and pharmaceutically acceptable salts thereof.

According to another aspect of the invention, there are also provided novel compounds of the formula (I) above, selected from:
3,5-dihydroxybenzylidene-malononitrile,
α-hydroxy-3,4,5-trihydroxybenzylidene-malononitrile,
α-cyano-3,4-dihydroxycinnamthioamide,
4-formylbenzylidene-malononitrile,
3,4-methylenedioxy-6-nitrobenzylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malonolnitrile,
γ-cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile; and pharmaceutically acceptable salts thereof.

The malonic acid derivatives of formula (I) above, can be prepared by known methods, for example by reacting a corresponding substituted benzaldehyde with malononitrile to obtain the benzylidene derivatives or with malononitrile dimers to obtain the cinnamylidene derivatives. The reaction is generally carried out in a suitable solvent, such as ethanol or benzene, and in the presence of a catalyst, e.g., piperidine, pyridine or β-alanine. Alternatively, a suitably substituted benzoyl chloride, e.g. triacetyl-galloyl chloride, can be reacted with malononitrile in the presence of an amine in a non-polar organic solvent.

The EGFR-inhibitor activity was tested on partially purified EGF receptors and on cell culture samples and the results are summarized in Table 1 herein.

In order to better understand the invention, reference will be made to the attached drawings, in which:

Fig. 1 is a graphical representation of the activity of isolated EGFR kinases (given in percent of the total kinase activity) plotted against the concentrations in μM of 12 different inhibitors.

Figs. 2a and 2b are graphical representations of the inhibitory effect of two pairs of tested compounds on the rate of the growth of KB and A431 cells, respectively, the number of cells being plotted against time (in days).

Fig. 3 is a graphical representation of the inhibition of A 431 cell growth as a function of various concentrations (in μM) of the inhibitor "compound 2" according to the invention.

Figs. 4a and 4b are graphical representations of the inhibitory effect of two pairs of tested compounds on the rate of the EGF dependent proliferation of A431/clone 15 cells. Fig. 4a depicts inhibition effects of compounds found to inhibit EGF dependent growth preferentially and Fig. 4b depicts inhibition effects of compounds found to inhibit EGF dependent growth exclusively.

The invention will now be described in more detail in the following non-limiting examples.

Preparative Examples

Example 1

3,4-Dihydroxybenzylidene-malononitrile

(Table 1, Compound 2)

To 11g (80 mM) of 3,4-dihydroxybenzaldehyde and 5.5g (83 mM) of malononitrile in 40 ml of ethanol, 7 drops of piperidine were added. The mixture was then heated at 70°C for 0.5-1 hour and then poured into water. The resulting solid precipitate was separated by filtration to give 12.7g (86% yield) of a yellow solid, 3,4-dihydroxybenzylidene-malononitrile, m.p.225.

Following the same procedure there were prepared:

3,5-dihydroxybenzylidene-malononitrile (Compound 1 in Table 1),

3-methoxy-4,5-dihydroxybenzylidene-malononitrile (Compound 3 in Table 1),

3,4,5-trihydroxybenzylidene-malononitrile (Compound 4 in Table 1).

3,5-di-t-butyl-4-hydroxybenzylidene-malononitrile,

3-hydroxybenzylidene-malononitrile.

Example 2

α-hydroxy-3,4,5-trihydroxybenzylidene-malononitrile

(Table 1, Compound 5)

To 2g (30mM) of malononitrile and 4 ml (40 mM) of triethylamine in 100 ml of CH_2Cl_2, triacetyl galloyl chloride (prepared from 7g (24 mM) of triacetyl gallic acid and thionyl chloride) in 50 ml CH_2Cl_2 was added. The resulting mixture was then stirred for 2 hours at room temperature, poured into 50 ml of water and hydrolyzed by heating for 2 minutes at 80°C with a solution of 2.5g of NaOH in 30 ml of ethanol. The mixture was then extracted with ethyl acetate and the organic extract was further worked up by washing with water, drying, filtering and evaporating it. Chromatography on silica gel gave 1.5g (29% yield) of α-hydroxy 3,4,5-trihydroxybenzylidene-malononitrile as an oily solid.

Example 3

α-cyano-3,4-dihydroxycinnamamide

(Table 1, Compound 6)

Reaction of 2.4g (10 mM) of 3,4-dihydroxybenzaldehyde and 0.9g (10.7 mM) of cyanoacetamide, by the procedure described in Example 1 above, gave 1.45g (70% yield) of α-cyano-3,4-dihydroxycinnamamide, as a yellow solid, m.p.247°C.

Example 4

γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile

(Table 1, Compound 7)

1.4g (10 mM) of 3,4-dihydroxybenzaldehyde, 1.4g (10.6 mM) of malononitrile dimer and 0.3g of β-alanine in 50 ml ethanol were heated at 70 °C for 40 minutes. 100 ml of water were added and the suspension was cooled and a solid precipitate was filtered off, washed with water and dried to give 1.3g of a yellow-orange solid, mp.235 °C, 53% yield, of γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile.

Following the same procedure there were prepared:
γ-Cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile (Compound 12 in Table 1), and
γ-Cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile (Compound 15 in Table 1).

Example 5

α-cyano-3,4-dihydroxycinnamic acid

(Table 1, Compound 8)

a) 2g (15 mM) of 3,4-dihydroxybenzaldehyde, 3g (21 mM) of t-butyl cyanoacetate and 0.5 ml of piperidine in 50 ml ethanol were heated to reflux for 1 hour. The resulting mixture was then poured into water and a solid precipitate was separated by filtration and was then washed and dried to yield 2.5g (yield 66%) of t-butyl α-cyano-3,4-dihydroxycinnamate as a yellow solid.

b) 1.6g of the t-butyl ester from a) in 10 ml of Trifluoro Acetic Acid was stirred at room temperature for 20 minutes. 50 ml of H_2O were added and the cooled suspension filtered to yield a solid precipitate which was washed with water and dried to give 1g (yield 85%) of α-cyano-3,4-dihydroxycinnamic acid as a yellow solid, mp.240 °C.

Example 6

α-cyano-3,4-dihydroxycinnamthioamide

(Table 1, Compound 9)

To 0.83g (6 mM) 3,4-dihydroxybenzaldehyde and 0.7g (7 mM) cyanothioacetamide in 30 ml ethanol were added 4 drops of piperidine. The mixture was refluxed for 1 hour and poured into ice-water. Filtering and drying gave 0.54 g, (41% yield), of an orange solid, mp.213 °C.

Anal. Calc. for $C_{10}H_8N_2O_2S$:	C = 54.54,	H = 3.64,	N = 12.73;
Found:	C = 54.44,	H = 3.87,	N = 12.91

MS: 220(M+,93%), 219(M-1,100%), 203(M-OH,26%), 186(M-2OH,24%), 123(33%), 110(30%), 100(43%), m/e.

Example 7

3,4-methylenedioxy-6-nitrobenzylidene malononitrile

(Table 1, Compound 11)

1g (5.1 mM) 3,4-methylenedioxy-6-nitrobenzaldehyde, 0.4 g (6 mM) malononitrile and 0.2 g β-alanine in 30 ml ethanol were stirred 16 hours at room temperature. 50 ml H_2O were added. Filtering gave 1g, (80% yield) of a bright yellow solid, mp.104 °C.
NMR (acetone-d_6): 6.42 (2H, s, methylenedioxy), 7.45 (1H, s, H_2), 7.82 (1H, s, H_5), 8.70 (1H, s, vinylic proton).
Following the same procedure there were also prepared:

γ-Cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile (Compound 13 in Table 1), and
γ-Cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malononitrile (Compound 14 in Table 1).

In Table 1 below there are listed 15 compounds according to the invention, 11 of which are new. All compounds gave correct analytical and spectroscopic data.

In this Table, KInh is the dissociation constant of the complex PTK-inhibitor and is expressed in μM units. The different KInh values were determined by the analysis according to Dixon.

Table 1

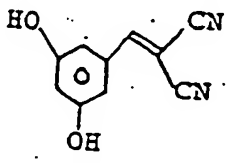
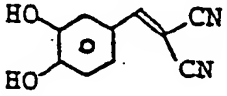
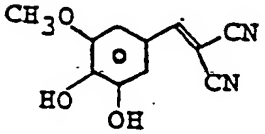
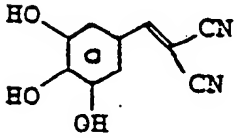
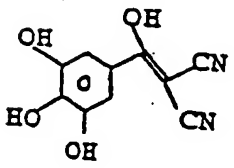
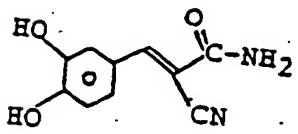
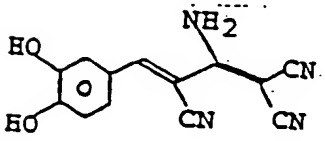
No.		mp, °C	KInh, μM	Literature
1		175	10	New
2		225	11±0.1	1*
3		235	2.2±0.3	New
4		245	1	1*
5		oil	4.5	New
6		247	3.5±0.6	1*
7		235	Non-competitive inhibitor	New

Table 1 (continued)

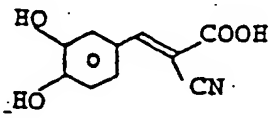
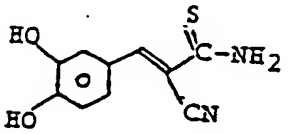
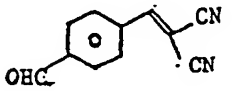
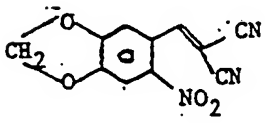
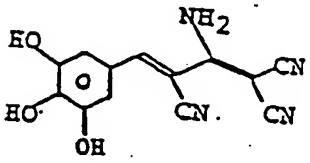
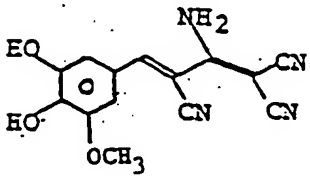
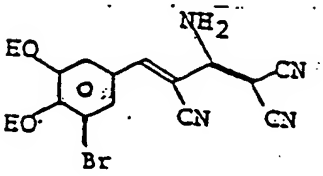
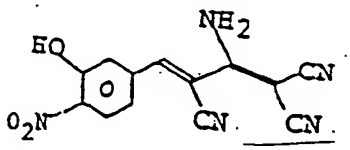
No.		mp,°C	KInh,µM	Literature
8		240	23.6	1*
9.		213	0.85	New
10.		142	20	New
11.		104	- **	New
12.		275	Non-competitive inhibitor	New
13.		225	Non-competitive inhibitor	New
14.		241	Non-competitive inhibitor	New

Table 1 (continued)

No.		mp, °C	KInh, µM	Literature
15.		219	Non-competitive inhibitor	New

* K.W. Rosemund and T. Boehm, ann. 437, 125 (1924).
** KInh was not calculated.

EGFR Inhibition Tests

Test on extracted EGF Receptors:

EGF receptors were prepared from A431 cells (obtained from the ATCC) and PTK activity of these receptors was assayed as described by S. Braun, W. E. Raymond and E. Racker, J. Biol. Chem. 259, 2051-2054 (1984). The compounds listed in Table 1 were tested for their inhibitory capacity on the EGF-receptor kinase activity, using the assay described above. Figure 1 demonstrates characteristic results using 10 inhibitors. The assay conditions were as described above using 0.125 mg of copoly $Glu^6Ala^3Tyr^1$. Dissociation constants were calculated from the inhibition curves and are listed in Table 1 above and indicated for each formula in Figure 1.

Tests on cells in tissue culture:

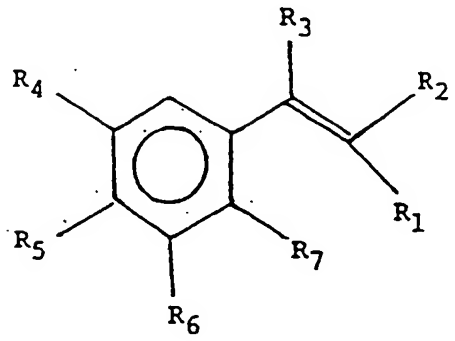
a) A431 cells and KB cells express EGF receptors on their cell surface and their growth rate depends on the presence of growth factors in the medium.
These cells were seeded and grown as described in O.Kashles and A.Levitzki, Bichem. Pharmacol., 35, 1531-1536 (1987). The compounds, the formulae of which are given in Fig. 2, were added to the medium at a cells concentration of Ca. $2x10^5$ cells/well. The inhibitor was added to the medium 1 hour after seeding. The medium volume in a well was 1 ml and the concentration of inhibitor therein 20 µM. Every 24 hours cells were counted and fresh medium with inhibitor applied to the remaining wells. The growth curves were determined in 24-well Costar dishes.

b) Some of the compounds according to the present invention are exclusive inhibitors to EGF dependent growth of cells and others are preferential inhibitors to such growth. Examples of the former are depicted in Fig. 4b and of the latter in Fig. 4a. In the experiment depicted in these Figures, 25,000 cells per well were placed in a 24 wells plate (Costar) supplied with Dulbeco medium containing 10% foetal calf serum, with 10 ng/ml EGF(●, ■, ▲) or with no added EGF (○, □ ,△). EGF receptor kinase inhibitors at various concentrations were added to the cells two hours after plating. The medium containing the inhibitors was replaced with fresh inhibitor containing medium every other day. On the fifth day, the number of cells in the presence of EGF and in the absence of EGF was determined. In Figs. 4a and 4b "100%" refers to the number of cells in the absence of inhibitor for each mode of cell growth (without EGF: 100,000 ± 10,000 cells; with EGF: 260,000 ± 30,000 cells for seven experiments). The filled symbols (●, ■, ▲) in Figs. 4a and 4b refer to inhibition of EGF stimulated growth, whereas open symbols (○, □, △) depict inhibition of EGF independent growth. Each experimental point represents the average of triplicate determination where the variance was less than 5 per cent. The compound numbers refer to compounds in Fig. 1.

The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

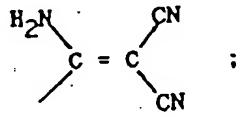
Claims

1. Pharmaceutical compositions containing as an active ingredient a compound of the general formula (I):



(I)

wherein one of R_1 and R_2 is CN and the other of R_1 and R_2 is $-C(X)NH_2$, in which X is O or S,
R_3 is H, CH_3 or OH,
R_4, R_5, R_6, R_7, are each independently H, OH, C_{1-5} alkyl, C_{1-5} alkoxy, NH_2, CHO, halogen, NO_2 or COOH, or R_4 and R_5 together may represent a group $-O-CH_2-O-$;
provided that: (a) when R_4 and R_7 are each OH, R_3, R_5 and R_6 are each H and one of R_1 and R_2 is CN, then the other of R_1 and R_2 cannot be $CONH_2$; and (b) when R_3 and R_7 are each H, R_5 is OH and R_4 and R_6 are both H or both C_{1-5} alkyl, then R_1 is CN and R_2 is CN or the group

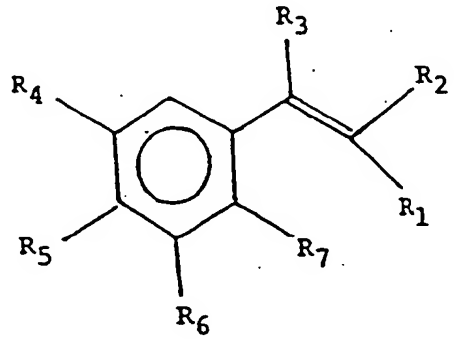


;

or a pharmaceutically acceptable salt thereof.

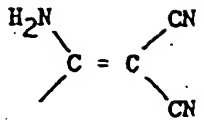
2. The use of a compound of the general formula I, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament.

3. Pharmaceutical compositions containing as an active ingredient a compound of the general formula (I):

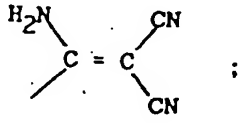


(I)

wherein R_1 and R_2 are each independently CN, $CONH_2$ or COOH or one of R_1 and R_2 may be $-CSNH_2$ or, when R_1 is CN, R_2 can also be the group



R_3 is H, CH_3 or OH,
R_4, R_5, R_6, R_7 are each independently H, OH, C_{1-5} alkyl, C_{1-5} alkoxy, NH_2, CHO, halogen, NO_2 or COOH, or R_4 and R_5 together may represent a group $-OCH_2-O-$;
provided that: (a) when R_4 and R_7 are each OH, R_3, R_5 and R_6 are each H and one of R_1 and R_2 is CN, then the other of R_1 and R_2 cannot be $CONH_2$; and (b) when R_3 and R_7 are each H, R_5 is OH and R_4 and R_6 are both H or both C_{1-5} alkyl, then R_1 is CN and R_2 is CN or the group



or a pharmaceutically acceptable salt thereof.

4. Pharmaceutical compositions according to Claim 3 comprising an active ingredient of formula I in which at one of R_1 and R_2 is CN cis to the phenyl moiety of said formula, or a pharmaceutically acceptable salt thereof.

5. Pharmaceutical compositions according to Claim 4 comprising an active ingredient in which R_4 and R_5 are hydroxy groups, R_6 is hydrogen or hydroxy and R_3 and R_7 are hydrogens, or a pharmaceutically acceptable salt thereof.

6. Pharmaceutical compositions according to Claim 3 containing as an active ingredient a compound selected from:
3,5-dihydroxybenzylidene-malononitrile,
α-hydroxy-(3,4,5- trihydroxybenzylidene)-malononitrile,
3-methoxy-4,5-dihydroxybenzylidene-malononitrile,
α-cyano-3,4-dihydroxycinnamthioamide,
α-cyano-3,4-dihydroxy-cinnamamide,
3,5-di-t-butyl-4-hydroxybenzylidene-malononitrile,
4-formylbenzylidene-malononitrile,
4-hydroxybenzylidene-malononitrile,
3,4-methylenedioxy-6-nitrobenzylidene-malononitrile,
3,4-dihydroxybenzylidene-malonitrile,
3,4,5-trihydroxybenzylidene-malonitrile,
γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malononitrile, and
γ-cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile;
and pharmaceutically acceptable salts thereof.

7. Novel compounds of formula (I) in Claim 3 and selected from:
3,5-dihydroxybenzylidene-malononitrile,
α-hydroxy-3,4,5-trihydroxybenzylidene-malononitrile,
3-methoxy-4,5-dihydroxybenzylidene-malononitrile,
α-cyano-3,4-dihydroxycinnamthioamide,
4-formylbenzylidene-malononitrile,
3,4-methylenedioxy-6-nitrobenzylidene-malononitrile,

γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile,
γ-cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malonolnitrile, and
γ-cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile;
and pharmaceutically acceptable salts thereof.

8. A process for the preparation of 3,5-dihydroxybenzylidene-malononitrile, 3-methoxy-4,5-dihydroxybenzylidene-malononitrile, 3,4,5-trihydroxybenzylidene-malononitrile, 3,4-methylendioxy-6-nitro-benzylidene-malononitrile, γ-cyano-β-amino-3,4-dihydroxy-5-methoxycinnamylidene-malononitrile, and γ-cyano-β-amino-3,4-dihydroxy-5-bromocinnamylidene-malononitrile which comprises reacting the corresponding substituted benzaldehyde with malononitrile in a polar organic solvent and in the presence of a suitable catalyst.

9. A process for the preparation of -hydroxy 3,4,5-trihydroxybenzylidene-malononitrile, which comprises reacting triacetyl galloyl chloride with malononitrile in the presence of an amine in a non-polar organic solvent, and hydrolyzing the product.

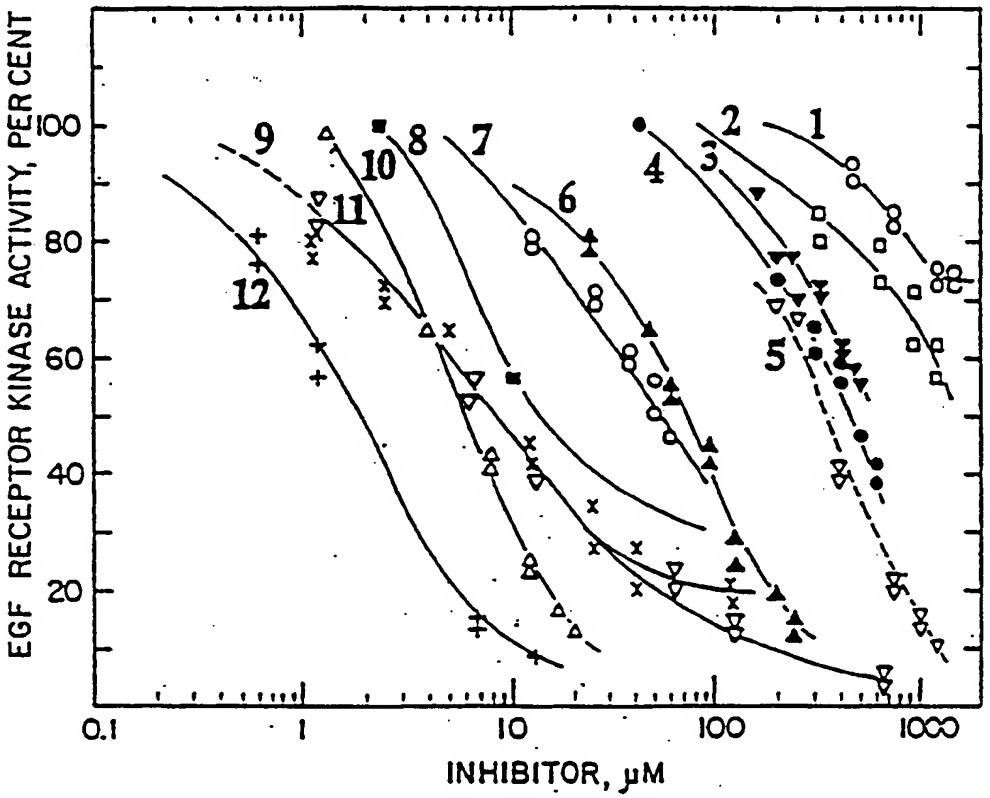
10. A process for the preparation of γ-cyano-β-amino-3,4-dihydroxycinnamylidene-malononitrile, γ-cyano-β-amino-3,4,5-trihydroxycinnamylidene-malononitrile and γ-cyano-β-amino-3-hydroxy-4-nitrocinnamylidene-malononitrile, which comprises reacting 3,4-dihydroxybenzaldehyde with malononitrile dimer in a polar organic solvent and in the presence of a suitable catalyst.

11. A process for the preparation of α-cyano-3,4-dihydroxycinnamthioamide which comprises reacting 3,4-dihydroxybenzaldehyde with cyanothioacetamide in the presence of a suitable catalyst.

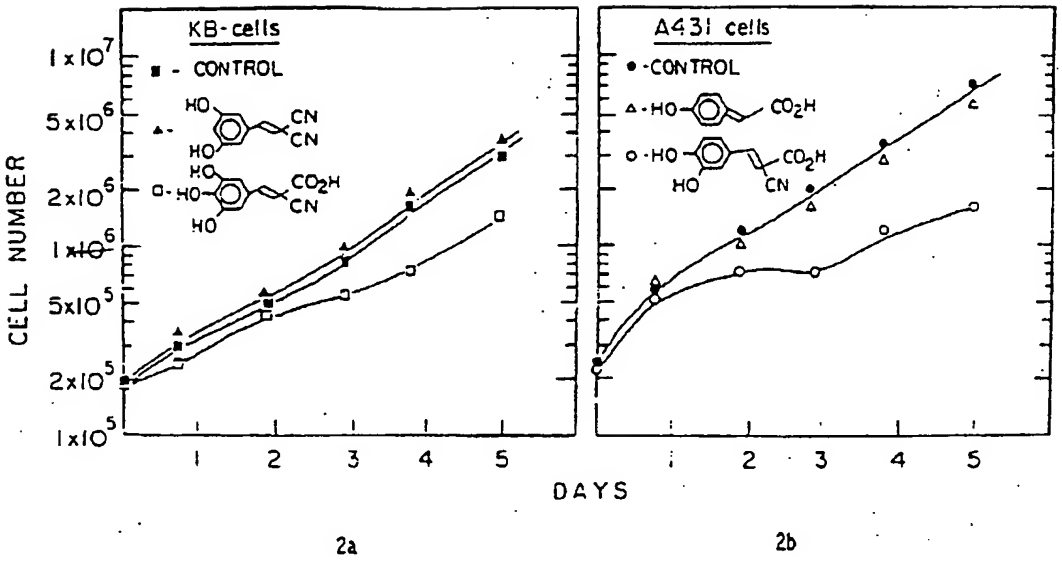
12. The use of compounds of formula (I) as defined in Claim 3 as specific protein-tyrosine kinase inhibitors.

FIGURE 1

INHIBITION OF EGF RECEPTOR KINASE BY ARYLIDENE DERIVATIVES



COMPOUND	SUBSTITUENTS							KInh μM
	R_3	R_4	R_5	R_6	R_7	R_2	R_1	
1	H	H	OH	H	H	CO_2H	H	1000
2	H	H	OH	H	H	CO_2H	CO_2H	500
3	H	H	OH	H	H	CN	CN	166
4	H	OH	OH	H	H	CO_2H	H	150
5	H	H	H	OH	H	CN	CN	123
6	H	OH	H	H	OH	CN	CO_2H	24
7	H	H	OH	OH	H	CO_2H	CN	18
8	H	H	OH	OH	H	CN	CN	11
9	H	OCH_3	OH	OH	H	CN	CN	2
10	H	OH	OH	OH	H	CN	CN	1
11	H	H	OH	OH	H	$CONH_2$	CN	2.3
12	H	H	OH	OH	H	$CSNH_2$	CN	0.85


KB-cells
■ - CONTROL
▲-
□-
A431 cells
● -CONTROL
△-
○-
CELL NUMBER
1x10^7
5x10^6
2x10^6
1x10^6
5x10^5
2x10^5
1x10^5
1
2
3
4
5
DAYS

2a 2b

Fig. 2

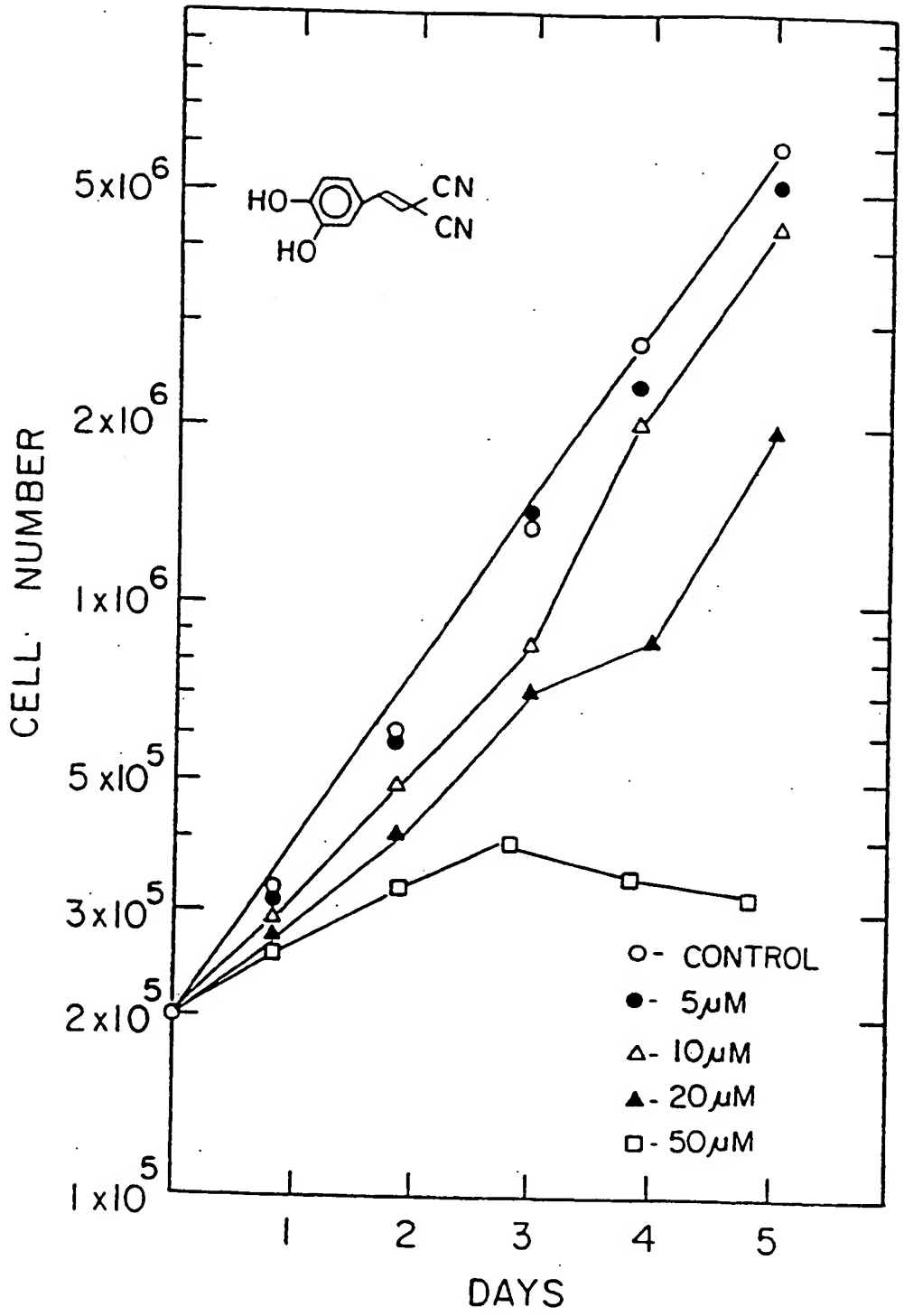
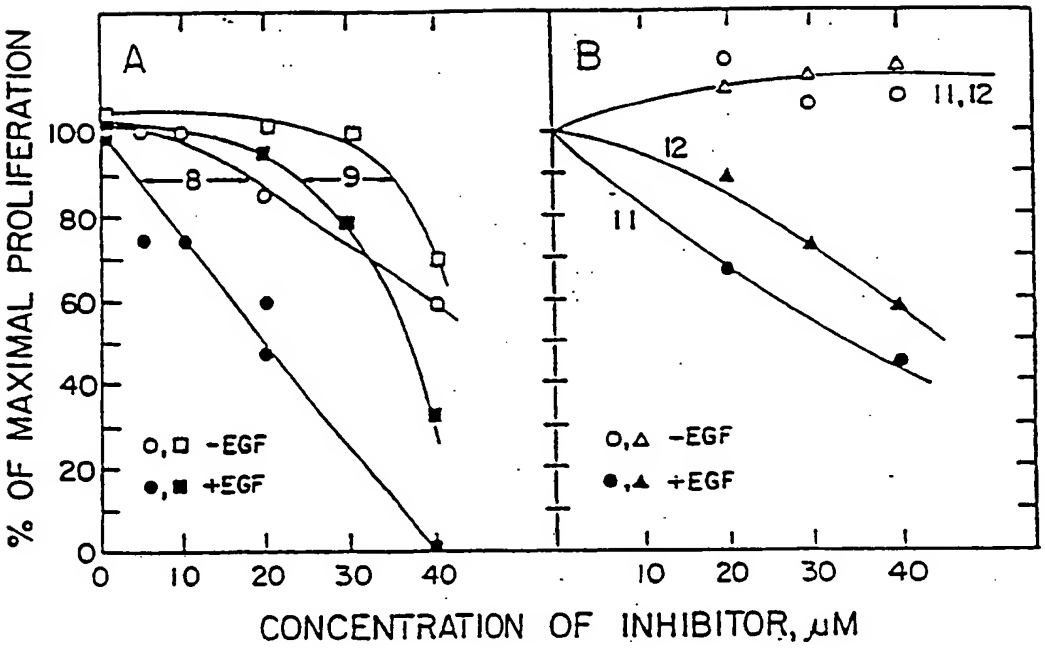

HO
HO
CN
CN
CELL NUMBER
5x10^6
2x10^6
1x10^6
5x10^5
3x10^5
2x10^5
1x10^5
1
2
3
4
5
DAYS
○- CONTROL
●- 5μM
△- 10μM
▲- 20μM
□- 50μM

Fig. 3

FIG. 4a

FIG. 4b


A
B
% OF MAXIMAL PROLIFERATION
100
80
60
40
20
0
0
10
20
30
40
8
9
1
11,12
12
11
o,□ -EGF
●,■ +EGF
o,△ -EGF
●,▲ +EGF
CONCENTRATION OF INHIBITOR, μM

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 614 661 A3**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **93119976.4**

(22) Date of filing: **21.12.88**

(51) Int. Cl.5: **A61K 31/165**, A61K 31/275, C07C 121/70, A61K 31/19, A61K 31/36, C07D 317/62

(30) Priority: **24.12.87 IL 8493787**
10.11.88 IL 8835488

(43) Date of publication of application:
14.09.94 Bulletin 94/37

(60) Publication number of the earlier application in accordance with Art.76 EPC: **0 322 738**

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

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02.11.94 Bulletin 94/44

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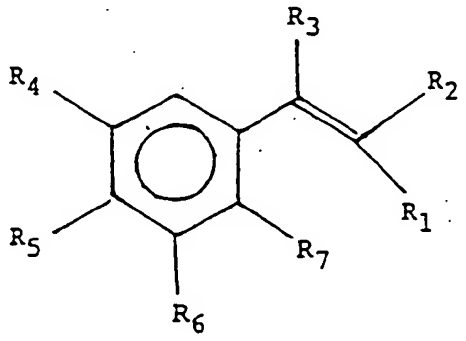
(54) Benzylidene- and cinnamylidine-malononitrile derivatives for the inhibition of proliferative processes in mammalian cells.

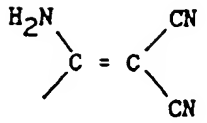
(57) There are provided pharmaceutical compositions containing as an active ingredient a compound of the general formula (I):



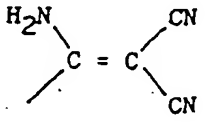
(I)

wherein R_1 and R_2 are each independently CN, $CONH_2$ or COOH or one of R_1 ad R_2 may be $-CSNH_2$ or, when R_1 is CN, R_2 can also be the group

EP 0 614 661 A3



R_3 is H, CH_3 or OH,
R_4, R_5, R_6, R_7 are each independently H, OH, C_{1-5} alkyl, C_{1-5} alkoxy, NH_2, CHO, halogen, NO_2 or COOH, or R_4 and R_5 together may represent a group -O-CH_2-O-;
provided that: (a) when R_4 ad R_7 are each OH, R_3, R_5 and R_6 are each H and one of R_1 and R_2 is CN, then the other of R_1 and R_2 cannot be $CONH_2$; and (b) when R_3 and R_7 are each H, R_5 is OH and R_4 and R_6 are both H or both C_{1-5} alkyl, then R_1 is CN and R_2 is CN or the group



;

or a pharmaceutically acceptable salt thereof.

There are also provided some novel compounds of formula (I) above.

The compositions and compounds according to the invention are efficient protein-tyrosine kinase inhibitors and are suitable for the inhibition of proliferative processes in mammalian cells.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 93 11 9976

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,X	DATABASE WPI Week 8713, Derwent Publications Ltd., London, GB; AN 87-089860 & JP-A-62 039 523 (KANEGAFUCHI CHEM KK) 20 February 1987 * abstract * ---	1-7,11, 12	A61K31/165 A61K31/275 C07C121/70 A61K31/19 A61K31/36 C07D317/62
P,X	SCIENCE, vol.242, 11 November 1988 pages 933 - 935 YAISH, PINNA ET AL 'BLOCKING OF EGF-DEPENDENT CELL PROLIFERATION BY EGF RECEPTOR KINASE INHIBITORS' * the whole document * * especially page 934, fig. 2, compounds 11 & 12 * --- -/--	1-7,11, 12	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61K
C07C
C07D

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	1 March 1994	MAIR J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0C07)

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.
namely:

see sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: mentioned in item 1.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 93 11 9976

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	CHIMIE THERAPEUTIQUE, vol.VIII, no.2, 1973 pages 188 - 193 DORE, J-C. ET AL 'CHIMIOTH RAPIE ANTITUMORALE ET SYNTHÈSES DANS LE DOMAINE DES ANTITUMORAUX D'ORIGINE NATURELLE. VII. D RIV S POLYNITROVINYLIQUES, BIS-BENZYLIDÈNE-AC TONES ET BIS-(DICYANO-2'2' VINYL)-1,4 BENZÈNE' * the whole document * ---	1-7,11, 12	
X	CHEMICAL AND PHARMACEUTICAL BULLETIN, vol.34, no.4, 1986 pages 1619 - 1627 KATSUMI, I. ET AL 'STUDIES ON STYRENE DERIVATIVES. II. SYNTHESIS AND ANTIINFLAMMATORY ACTIVITY OF 3,5-DI-TERT-BUTYL-4-HYDROXYSTYRENES'	1-7	TECHNICAL FIELDS SEARCHED (Int.Cl.4)
A	* the whole document * * especially page 1623, table I, compound no. 29 * ---	11,12	
A	US-A-4 064 266 (BIRCHALL, G.R. ET AL) 20 December 1977 * the whole document * -----	1-7,11, 12	

EPO FORM 1503 03.82 (P04C19)

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

Since the scope of the subject matter of this divisional application is just as broad as that of the parent application (Cf. claim 1 of the original and claim 3 of the divisional application), the lack of unity a posteriori observed in the original application must inevitably apply to this divisional application. However, since the claims are differently presented the first replacing subject (on which the search has been performed) is also different.
The application has been divided into the following subjects:

1. Claims 1,11 (completely); 2,3-7,12 (partially) :

   Pharmaceutical compositions containing compounds of formula I wherein one of R1 and R2 is -CN and the other is -C(X)NH2 wherein X is O or S (including novel compounds and processes for their preparation) and their use for the treatment of cancer.

2. Claim 9 (completely); 2-8,12 (partially) :

   Pharmaceutical compositions containing compounds of formula I wherein R1 and R2 are both -CN (including novel compounds and processes for their preparation) and their use for the treatment of cancer.

3. Claims 2-5,12 (partially) :

   Pharmaceutical compositions containing compounds of formula I wherein one of R1 and R2 is -CN and the other is -COOH, and their use for the treatment of cancer.

4. Claim 10 (completely); 2-8,12 (partially) :

   Pharmaceutical compositions containing compounds of formula I wherein R1 is -CN and R2 is the group -C(NH2)=C(CN)2 (including novel compounds and processes for their preparation) and their use for the treatment of cancer.

5. Claim 3 (partially) :

   Pharmaceutical compositions containing compounds of formula I wherein R1 and R2 are each independently -CONH2 or -COOH or one of R1 and R2 is -CSNH2 and the other is -CONH2 or -COOH and their use for the treatment of cancer.

Only the first subject has been searched.

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INCOMPLETE SEARCH

Claims searched incompletely : 1

Reason : In claim 1 proviso B) (see page 14, lines 11-14) appears to contain contradictions to the rest of the claim. The first requirement of claim 1 is that one of R1 and R2 is -CN and the other is -C(X)NH2 in which X is O or S. Proviso B) requires that in certain cases both R1 and R2 should be -CN which is clearly not compatible with the first requirement. Alternatively proviso B) requires that in certain cases R1 should be -CN and R2 should be the group -C(NH2)=C(CN)2 which is not even mentioned in the first requirement. For these reasons proviso B) was ignored and the search was based on the rest of claim 1.